

The University of Chicago

BLOOD CHOLINE VARIATIONS IN
PREGNANCY, IN LABOR, AND
IN THE PUERPERIUM

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
1940

By
EDWARD EAGLE

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BLOOD CHOLINE VARIATIONS IN PREGNANCY, IN LABOR, AND IN THE PUERPERIUM

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REVIEW OF LITERATURE

THE first extraction of a physiologically active substance from the human placenta was made in 1885 by Böhm,¹ who found an average of 50 mg. of choline per placenta.

Twenty-two years later Rieländer² likewise obtained approximately the same result (average 40 mg. of choline per placenta). In the same year (1907), Dixon and Taylor³ reported that the placenta produces a chemical substance which develops with the ripening of that tissue, the liberation of which by the contracting uterus and vessels might induce the onset of normal labor. In 1928, Sievers⁴ confirmed the previously reported choline content of the placenta (average 40-45 mg. per organ) and proposed the theory that the latter tissue stored choline which was transferred to the uterus by pressure on the placenta at the time of birth, thus causing the uterine contractions. Larger amounts of choline in the placenta were reported by other authors (5.0-120.0,⁵ 61.0-92.2,⁶ 51.5-243.0,⁷ 54.0-163.0,⁸ 6.0-84.0,⁹ 0-945.0,¹⁰ and 102.0-282.0¹¹). Smyth¹² has recognized a water-soluble precursor of choline in the human placenta, each molecule of which contains one molecule of choline, one of phosphoric acid, and one amino linkage.

Hauptstein¹³ concluded that if choline were to be considered responsible for the uterine contractions during labor, it must be present as the far more powerful acetic ester, acetylcholine, and noted that in 18 cases the placenta contained acetylcholine in amount varying from 74 to 459 mg. per kg. of tissue. From then on, Bischoff and others;⁹ Chang and Wong;¹⁴ Wong and Chang;¹⁵ and Wen, Chang and Wong,¹⁶ who found the origin of the free placental acetylcholine to be in the syncytial layer of the villus, reported the following acetylcholine values, respectively: 168-220, 15-133.3, 12.5-133.3, and 19-104 mg. per kg. of tissue. Chang^{17, 18} reported (1935) that there is a reserve of acetylcholine in the placenta which may be liberated from its precursor in vitro, and found (1938) that there is a "free" and a "bound" fraction of acetylcholine in the human placenta. Hunt¹⁹ pointed out many years ago that intravenous injection in cats of as little as 0.000,000,0024 mg. of acetylcholine per kg. body weight can cause a pronounced fall in blood pressure. The presence in the placenta of such large amounts of this powerful substance may be more than a coincidence.

That the uterus responds to choline by contraction has been known for some time, particularly since the reports of Müller,²⁰ Adler²¹ and Berlin²² who pointed out that the effect of choline on the uterus of the cat or mouse simulated the contractions in labor. Engeland and Kutscher²³ have reported that choline causes tetanic contractions of the gravid uterus, and Alberts²⁴ has stated that the gravid uterus is more sensitive to choline than the nongravid one. In a study of the quantitative action of acetylcholine on the guinea pig uterus, Webster²⁵ found that the height of contraction varies with the concentration, but even a molar concentration of 4.4×10^{-7} gave good contractions. Sherif²⁶ showed that acetylcholine acts as the chemical transmitter of the effects of the hypogastric nerves on the uterine muscle, similar to that of the vagi nerves.

The effectiveness of acetylcholine as an oxytocic agent was recently studied in a series of 28 patients.¹⁵ In 7 out of 16 cases in which the membranes were intact,

acetylcholine induced labor and brought about delivery; in the other 9 cases uterine contractions were obtained but did not last long enough to terminate pregnancy. The procedure was successful in inducing labor in all of the 12 cases where the membranes were ruptured spontaneously or artificially, and rhythmic uterine contractions were initiated within a few minutes regardless of whether the patient was a primipara or a multipara.¹⁵ Contractions of the guinea pig uterus after application of blood from women in labor had been noted by Brdiczka²⁷ and Fontes,²⁸ and within recent years considerable attention has been directed to the presence of some oxytocic material in the blood of women in labor.

Prior to 1923 most investigators were unable to find free choline in the blood or serum of man or animals. From 1923 to the present the values reported were zero in six instances and in twenty-four others the amounts have ranged from 0.5 to 310.0 mg. per liter. The lowest values on human blood (aside from zero) were obtained by Kahlson²⁹ (2.2-8.5 mg./liter), and the highest by Vasiliiu³⁰ (18.2-147.0 mg./liter). The inaccuracy and nonspecificity of the methods employed can account for such wide divergence in results. Some of these methods are discussed in connection with a study of the choline content of biologic fluids.³¹

METHOD

The method used in the present study has been described in detail elsewhere.³¹ Blood choline determinations were made on venous (arm) blood of women in various stages of pregnancy, labor, and the puerperium at the Chicago Lying-in Hospital or its "Stockyards Dispensary." The analyses were done in duplicate; in some cases even three

TABLE I. BLOOD CHOLINE VALUES DURING PREGNANCY

NO.	AGE YEARS	OBSTET. HISTORY*	DAYS BE- FORE DE- LIVERY	CHOLINE FOUND MG./LITER	NO.	AGE YEARS	OBSTET. HISTORY*	DAYS BE- FORE DE- LIVERY	CHOLINE FOUND MG./LITER
1	22	0,0,0,0	0	12.1	32	27	0,0,0,0	40	15.2
2	19	0,0,0,0	1	15.8	33	23	0,0,0,0	41	11.6
3	22	0,0,0,0	1	10.9	34	43	1,0,0,1	41	13.2
4	26	0,0,0,0	1	15.6	35	31	2,0,0,2	54	13.0
5	24	3,0,3,0	3	10.4	36	34	0,0,0,0	55	11.4
6	25	1,0,0,1	4	10.0	37	29	2,0,0,2	55	16.2
7	18	1,0,1,0	4	13.2	38	32	6,0,2,4	61	11.5
8	27	1,0,0,1	5	12.6	39	19	1,0,0,1	61	13.6
9	21	1,0,0,1	7	12.8	40	35	4,0,2,2	62	12.0
10	24	0,0,0,0	7	12.1	41	28	1,0,0,1	66	12.7
11	18	0,0,0,0	9	12.4	42	29	5,0,0,5	67	11.3
12	28	3,0,0,3	9	14.4	43	25	0,0,0,0	68	10.6
13	23	2,0,0,3	10	12.6	44	24	2,0,0,2	72	14.6
14	22	0,0,0,0	10	11.2	45	18	0,0,0,0	79	13.8
15	23	2,0,0,1	11	11.9	46	41	11,0,0,11	83	9.9
16	28	0,0,0,0	11	19.0	47	23	2,0,0,2	85	13.7
17	30	1,0,0,1	12	12.8	48	27	0,0,0,0	90	12.3
18	21	0,0,0,0	16	15.0	49	19	0,0,0,0	91	10.2
19	38	2,0,0,2	16	13.6	50	31	1,0,0,1	94	10.6
20	23	0,0,0,0	17	14.4	51	35	5,0,0,5	98	19.9
21	26	0,0,0,0	20	12.9	52	21	1,0,0,1	100	10.6
22	27	0,0,0,0	21	12.0	53	24	1,0,0,1	114	11.1
23	19	1,0,0,1	23	13.3	54	31	2,0,0,2	120	14.4
24	23	0,0,0,0	24	12.9	55	22	0,0,0,0	125	16.3
25	31	6,0,0,6	25	11.2	56	22	1,0,0,1	145	14.0
26	21	0,0,0,0	26	15.0	57	21	2,0,0,1	150	15.8
27	23	0,0,0,0	26	11.4	58	27	3,0,0,3	172	12.0
28	27	0,0,0,0	28	16.4	59	23	3,0,1,2	176	12.8
29	28	0,0,0,0	32	11.6	60	24	6,0,5,1	178	13.1
30	23	0,0,0,0	32	11.8	61	22	0,0,0,0	179	14.5
31	27	0,0,0,0	37	14.1	62	22	1,0,0,1	207	13.3

*Previous pregnancies, premature labors, abortions, and children now living.

or four analyses were performed on the same sample. Frequent blank determinations and control analyses were likewise performed. With but few exceptions all of the 87 experimental subjects were in good health. Some of the 18 male and 19 nonpregnant female controls were subsequently admitted to Billings Hospital from the Out-Patient Department (where the latter samples were obtained), but none was gravely ill or in acute distress at the time the blood sample was taken.

RESULTS

Blood Choline in Pregnancy.—Table I records the choline values on venous blood samples taken from 62 subjects at times varying from just prior to the onset of labor to 207 days before delivery. It may be seen that from the third lunar month of pregnancy (197-224 days before delivery) to term there was no significant variation in the average choline values, which for the third to the tenth lunar month inclusive were 13.3, 13.1, 14.9, 13.9, 12.9, 12.2, 13.1, and 13.1 mg. per liter, respectively. The average choline results for 28 primigravidas, 14 secundigravidas, and 20 multiparas were, respectively, 13.3, 12.4, and 13.2 mg. per liter; the grand average for all 62 subjects was 13.1 mg. of choline per liter of blood.

Blood Choline During Labor and Post Partum.—Table II summarizes the data on 17 subjects in various stages of labor. The average choline value for the entire group was 15.0 mg. per liter, somewhat higher than that for blood during pregnancy. Table III shows the post-partum choline results on 8 subjects. In 6 cases in which

TABLE II. BLOOD CHOLINE VALUES IN LABOR

NO.	AGE YEARS	OB- STET. HIS- TORY ^a	TIME SAMPLE HR.-MIN.	DURATION OF LABOR				DUR. OF PREG. WK.	BABY		CHO- LINE FOUND MG./ LITER	DE- LIV- ERY
				STAGES			TOTAL HR.- MIN.		WT. GM.	SEX		
				1ST HR.- MIN.	2ND HR.- MIN.	3RD HR.- MIN.						
63	26	1,0,1,0	6-10 ^b				24-30	40	e	M	13.5	e
64	24	0,0,0,0	6-11 ^b	13-45	0-51	0-7	14-43	39	3630	M	18.2	f
65	18	0,0,0,0	7-0 ^b	18-40	1-15	0-3	19-58	40	4150	F	13.6	g
66	23	2,0,0,2	7-0 ^b	9-35	0-10	0-15	10-0	40	2800	F	12.8	h
67	30	0,0,0,0	7-10 ^b	15-0	1-17	0-8	16-25	40	3025	M	21.2	i
68	20	0,0,0,0	7-30 ^b	14-35	1-16	0-9	16-0	40	3255	F	11.7	j
69	20	0,0,0,0	8-17 ^b	14-50	1-53	0-8	16-51	40	3590	F	18.3	j
70	28	3,0,0,2	8-55 ^b	11-5	0-22	0-8	11-35	40	3265	F	14.3	h
71	19	0,0,0,0	9-0 ^b	29-0	1-35	0-12	29-47	40	3360	M	12.4	g
72	34	3,0,0,3	9-3 ^b	11-30	0-31	0-10	12-11	40	4570	M	14.1	h
73	40	5,0,2,3	11-8 ^b	11-15	0-11	0-4	11-30	40	3840	M	13.7	h
74	28	0,0,0,0	11-43 ^b	27-15	1-8	0-7	28-30	40	3375	M	22.8	g
75	23	0,0,0,0	19-35 ^b	23-55	0-29	0-6	24-30	40	3245	M	10.7	j
76	27	1,0,0,1	25-58 ^b	41-0	0-6	0-4	41-10	41	3955	M	12.2	k
77	27	2,0,1,1	Onset ^c	18-30	0-58	0-6	19-44	40	3175	F	15.0	j
78	16	0,0,0,0	^d	20-55	1-25	0-2	22-22	38	3460	F	11.9	l
79	25	2,0,2,0	End ^c	7-15	0-37	0-5	7-58	40	3230	F	19.3	g

^aPrevious pregnancies, premature labors, abortions, children now living.

^bAfter onset of first stage of labor.

^cSecond stage of labor.

^dSeven minutes after onset of second stage of labor.

^eTwins: I, midforceps, 2,390 Gm., M.; II, breech extraction, 2,375 Gm., M. Acute fulminating toxemia; five convulsions during delivery; placenta removed manually.

^fForceps rotation; low forceps with episiotomy.

^gSpontaneous delivery with episiotomy.

^hSpontaneous delivery.

ⁱMild toxemia; spontaneous delivery with episiotomy.

^jLow forceps with episiotomy.

^kMidforceps with episiotomy.

^lManual rotation; low forceps with episiotomy.

TABLE III.—POST-PARTUM CHOLINE VALUES

NO.	AGE YEARS	OBSTET. HIS- TORY ^a	SAMPLE: TIME AFTER DELIV- ERY HR.-MIN.	DURATION OF LABOR				DUR. OF PREG. WK.	BABY		CHO- LINE FOUND MG./ LITER	DE- LIV- ERY
				STAGES			TOTAL HR.- MIN.		WT. GRAMS	SEX		
				1ST	2ND.	3RD						
				HR.- MIN.	HR.- MIN.	HR.- MIN.						
80	25	2,0,2,0	0-10	7-15	0-37	0-6	7-58	40	3230	F	24.9	b
81	18	0,0,0,0	0-10	33-35	1-35	0-13	34-10	40	2970	F	22.9	b
82	21	0,0,0,0	1-16	7-30	0-34	0-21	8-25	41	3095	M	19.0	b
83	18	0,0,0,0	1-17	5-0	1-51	0-2	6-53	40	2575	F	30.7	c
84	33	1,0,1,0	2-10	38-53	1-20	0-8	40-21	36	3040	F	25.9	e
85	34	10,0,0,10	4-15	8-15	0-14	0-7	8-36	41	3520	F	24.0	f
86	27	5,0,0,5	44 days	----	---	---	2-10	40	3859	M	13.4	d
87	33	6,0,0,5	48 days	----	---	---	6-0	40	4545	F	11.3	f

^aPrevious pregnancies, premature labors, abortions, children now living.

^bSpontaneous delivery with episiotomy.

^cManual rotation; spontaneous delivery with episiotomy.

^dUnattended labor.

^eMidforceps with episiotomy.

^fSpontaneous delivery.

blood was taken within 255 minutes after delivery, the average choline content was 24.6 mg. per liter. The significance of this marked rise in the choline content of maternal (venous) blood immediately after delivery of the placenta is not fully understood, and its explanation must await further knowledge of the role in the organism of choline and its esters which already enjoy unique distinction as a hormone and an essential food factor. Two subjects who were forty-four and forty-eight days post partum showed low or normal blood choline values (13.4 and 11.3 mg./liter).

The average choline content of the venous blood of 18 male subjects was 13.4 mg. per liter; that for 19 nonpregnant female subjects was 14.6 mg. per liter. The ages and diagnoses on these 37 individuals varied widely.

Neither in pregnancy, in labor, nor in the puerperium was there any obvious correlation between the choline content of the blood and the previous obstetric history, the age of the mother, the total duration of pregnancy, the duration of the various stages of labor, the weight or sex of the child, the weight of the placenta or the manner of delivery.

COMMENT

In 1932 Jeffcoate³² proposed the theory that estrin is essential for parturition, an idea which has been supported by the observations that estrin causes contractions of uterine muscle,³³⁻³⁵ and that estrin is present in considerable quantity during pregnancy, reaching its peak at the time of parturition.³⁶ Witherspoon³⁷ suggested that an inhibitory action on estrin (by the luteinizing factor in the ovary, placenta and anterior lobe of the hypophysis) which is present during early pregnancy is released toward the end of gestation, and an increased sensitivity of the uterus to pituitrin produces uterine contractions until delivery occurs. A theory involving the excitatory action of some hormone on the numerous ganglia of the uterus leading to contraction of that organ was advanced by Gibbons³⁸ who indicated that there remained only the discovery of something sufficiently powerful to excite the uterine ganglia to produce these essential contractions. The recent observation that the acetylcholine content of the uterus significantly increased within an hour after injection of estrin and completely disappeared after twelve hours is of interest in this connection.³⁹

In a study of the choline content of the saliva during the menstrual cycle, Eagle³¹ found that the choline value showed a cyclic variation, the maximum deviations from the lowest level for each of four subjects being 396, 294, 226, and 398 per cent, respectively. The choline curves showed peaks occurring on the second to seventh day, on the thirteenth to sixteenth day, and on the twenty-second to twenty-fourth day of the cycle, all of which are periods of considerable uterine activity. The results in the present study likewise indicate an elevated choline content of the blood (immediately following the delivery of the placenta) at a period of great uterine activity.

It will be recalled that choline and acetylcholine are normal constituents of the body; that the latter, which is recognized as the chemical transmitter of the effects of the hypogastric nerve on uterine muscle, is a powerful oxytocic agent causing rhythmical contractions of the uterus in man and animals; that the gravid uterus is more sensitive to choline and acetylcholine than the nongravid one; that the acetylcholine content of the uterus may be increased by estrin injection; and that the choline content of the blood and saliva is markedly elevated during periods of great uterine activity. On the basis of the foregoing, it is concluded that, whatever be the cause or causes of the onset of labor, the possible role of choline and its powerful esters in facilitating the emptying of the uterus at term should be considered.

SUMMARY

1. Choline determinations were made on blood samples from 62 women in various stages of pregnancy, 17 women in labor, 8 women in the puerperium, 19 nonpregnant females, and 18 males, a total of 124 samples.

2. The choline content of the blood from pregnant women is fairly constant from 207 days before delivery to term (average 13.1 mg./liter).

3. The blood choline value is slightly increased during labor (average 15.0 mg./liter).

4. The choline level is highest just after delivery (average 24.6 mg./liter). In two cases which were 44 and 48 days post partum, the choline results were 13.4 and 11.3 mg. per liter.

5. An average of 14.6 mg./liter was obtained for the choline content of blood from nonpregnant female patients; that for male patients was 13.4 mg. per liter.

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